

- 29 -

Claims

1. A modified recombinant host cell, which, in unmodified form, does not produce polyketides, which cell is modified to contain an expression system for a
5 minimal polyketide synthase (PKS) and an expression system for a holo ACP synthase,
said minimal PKS comprising a ketosynthase/acyl transferase (KS/AT) catalytic region, a chain-length factor (CLF) catalytic region and an acyl carrier protein (ACP) activity for an aromatic PKS; or
said minimal PKS comprising a KS catalytic region, an AT catalytic region, and
10 an ACP activity for a modular PKS or a fungal PKS.
2. The modified cell of claim 1 which is *E. coli* or yeast.
3. The modified cell of claim 1 wherein said PKS is the synthase for 6-
15 methyl salicylic acid.
4. The modified cell of claim 1 wherein the nucleotide sequence encoding said holo ACP synthase and the nucleotide sequence encoding at least a portion of said minimal PKS are fused so as to encode a fusion protein.
20
5. The modified cell of claim 1 wherein said expression system for said minimal PKS and said expression system for said holo ACP synthase are present on separate vectors.

- 30 -

6. The modified cell of claim 1 wherein at least one of said expression systems is integrated into the host cell chromosome.

7. A method to produce a polyketide which method comprises culturing the
5 cells of claim 1 under conditions wherein said expression systems produce the encoded proteins and wherein said polyketide is synthesized.

8. A recombinant host cell modified to contain either

a) at least two vectors; said first vector containing a first selectable marker
10 and a first expression system and said second vector containing a second selectable marker and a second expression system and optionally additional vectors containing additional selectable markers and expression systems wherein said expression systems contained on said vectors are effective to produce at least a minimal polyketide synthase (PKS); or

b) at least one vector and a modified chromosome, said one vector containing
15 a first selectable marker and a first expression system and said modified chromosome containing a second expression system and optionally additional vectors containing additional selectable markers and expression systems wherein said expression systems contained on said vectors in combination with said expression system on said
20 chromosome are effective to produce at least a minimal PKS;

said minimal PKS comprising a ketosynthase/acyl transferase (KS/AT) catalytic region, a chain-length factor (CLF) catalytic region and an acyl carrier protein (ACP) activity for an aromatic PKS; or

said minimal PKS comprising a KS catalytic region, an AT catalytic region, and
25 an ACP activity for a modular PKS.

- 31 -

9. The cell of claim 8 which is a yeast cell, an *E. coli* cell, an actinomycete cell or a plant cell.

10. The cell of claim 8 which further contains an expression system for a cell-based detection system for a functional polyketide.

11. The cell of claim 8 which produces at least a minimal aromatic PKS and which contains:

(a) a first vector comprising a first selectable marker and an expression system comprising a nucleotide sequence encoding a KS/AT catalytic region operably linked to a promoter operable in said cell;

(b) a second vector comprising a second selectable marker and an expression system comprising a nucleotide sequence encoding a CLF catalytic region operably linked to a promoter operable in said cell; and

(c) a third vector containing a third selectable marker and an expression system which comprises a nucleotide sequence encoding an ACP activity operably linked to a promoter operable in said cell.

12. The cell of claim 8 which produces at least a minimal modular PKS and which contains

(a) a first vector containing a first selectable marker and an expression system for at least one module of a polyketide synthase (PKS) operably linked to a promoter operable in said cell; and

(b) a second vector containing a second selectable marker and a nucleotide sequence encoding at least a second module of a polyketide synthase operably linked to a promoter operable in said cell.

- 32 -

13. The cell of claim 12 wherein said first and second module are derived from different polyketide synthases.

5 14. The cell of claim 13 wherein said nucleotide sequence encoding at least one module further contains a nucleotide sequence encoding a KR activity; or
wherein the nucleotide sequence encoding at least one module encodes a KR and DH activity; or

wherein said nucleotide sequence encoding at least one module encodes a KR,
10 DH and ER activity; and/or

wherein said nucleotide sequence encoding at least one module encodes a thioesterase (TE) activity.

15 15. A method to produce a polyketide which method comprises culturing the cells of claim 8 under conditions wherein said expression systems produce the encoded proteins and wherein said polyketide is synthesized.

16. The cell of claim 8 which is further modified to contain a recombinant expression system for a holo ACP synthase.

20

17. A method to produce a polyketide which method comprises culturing the cells of claim 16 under conditions wherein said expression systems produce the encoded proteins and wherein said polyketide is synthesized.

- 33 -

18. A library of polyketide synthases PKS or synthesized polyketides which comprises a panel of individual colonies, each colony containing either

- 5 a) at least two vectors; said first vector containing a first selectable marker and a first expression system and said second vector containing a second selectable marker and a second expression system and optionally additional vectors containing additional selectable markers and expression systems wherein said expression systems contained on said vectors are effective to produce at least a minimal polyketide synthase (PKS), or
- 10 b) at least one vector and a modified chromosome, said one vector containing a first selectable marker and a first expression system and said modified chromosome containing a second expression system and optionally additional vectors containing additional selectable markers and expression systems wherein said expression systems contained on said vectors in combination with said expression system on said chromosome are effective to produce at least a minimal PKS;

15 said minimal PKS comprising a ketosynthase/acyl transferase (KS/AT) catalytic region, a chain-length factor (CLF) catalytic region and an acyl carrier protein (ACP) activity for an aromatic PKS; and

said minimal PKS comprising a KS catalytic region, an AT catalytic region, and an ACP activity for a modular PKS

20 wherein the combination of vectors or of vector(s) and modified chromosome is different in each colony.

19. The library of claim 18 wherein said colonies are colonies of yeast, *E. coli*, actinomycetes or plant cells.

25

20. The library of claim 18 wherein each colony further contains an expression system for a cell-based detection system for a functional polyketide.

- 34 -

21. The library of claim 18 wherein the PKS are aromatic PKS and each colony contains:

5 (a) a first vector comprising a first selectable marker and an expression system comprising a nucleotide sequence encoding a KS/AT catalytic region operably linked to a promoter operable in said cell;

(b) a second vector comprising a second selectable marker and an expression system comprising a nucleotide sequence encoding a CLF catalytic domain operably linked to a promoter operable in said cell.

10 (c) a third vector containing a third selectable marker and an expression system which comprises a nucleotide sequence encoding an ACP activity operably linked to a promoter operable in said cell;

wherein said combination of first, second and third vectors is different in each colony.

15

22. The library of claim 18 wherein the PKS are modular PKS wherein each colony contains

a first vector containing a first selectable marker and an expression for at least one module of a PKS operably linked to a promoter operable in said cell; and

20 a second vector containing a second selectable marker and a nucleotide sequence encoding at least a second module of a polyketide synthase operably linked to a promoter operable in said cell;

wherein said combination of first and second vectors is different in each colony.

25 23. The library of claim 22 wherein said nucleotide sequence encoding at least one module further contains a nucleotide sequence encoding a KR activity; or

- 35 -

wherein the nucleotide sequence encoding at least one module encodes a KR and DH activity; or

wherein said nucleotide sequence encoding at least one module encodes a KR, DH and ER activity; and/or

5 wherein said nucleotide sequence encoding at least one module encodes a thioesterase (TE) activity.

24. The library of claim 18 wherein each colony further contains a recombinant expression system for a holo ACP synthase.

10

25. A method to produce a library of polyketides which method comprises culturing the cells of claim 18 under conditions wherein said expression systems produce the encoded proteins and wherein said polyketide is synthesized.

15

26. A method to produce a library of polyketides which method comprises culturing the cells of claim 24 under conditions wherein said expression systems produce the encoded proteins and wherein said polyketide is synthesized.

27. A method to identify a polyketide that binds a target receptor which
20 method comprises contacting said receptor with each member of the library of claim 18 under conditions wherein binding to said receptor can be detected; and

detecting the presence or absence of binding to said receptor with respect to each member, whereby

a member that binds to a receptor is identified.

25

- 36 -

28. A method to identify a polyketide that binds a target receptor which method comprises contacting said receptor with each member of the library of claim 24 under conditions wherein binding to said receptor can be detected; and

5 detecting the presence or absence of binding to said receptor with respect to each member, whereby

a member that binds to a receptor is identified.

29. A method to identify a polyketide functional in a cell-based detection system which method comprises assessing each member of the library of claim 18

10 for the presence or absence of signal in said cell-based detection system whereby a functional polyketide is identified.

30. A vector adapted for expression in yeast which vector contains a selectable marker operable in yeast, and an expression system which comprises the
15 coding region of at least one functional polyketide synthase catalytic activity operably linked to a promoter operable in yeast.

31. A yeast cell modified to contain the vector of claim 30.

20 32. The yeast cell of claim 31 which further contains a recombinant expression system for a holo ACP synthase.

33. A method to produce a polyketide synthase activity which method comprises culturing the yeast cell of claim 31 under conditions wherein expression is
25 favored.

- 37 -

34. A method to produce a polyketide synthase activity which method comprises culturing the yeast cell of claim 32 under conditions wherein expression is favored.

5

35. A vector adapted for expression in *E. coli* which vector contains a selectable marker operable in *E. coli*, and an expression system which comprises the coding region of at least one functional polyketide synthase catalytic activity operably linked to a promoter operable in *E. coli*.

10

36. An *E. coli* cell modified to contain the vector of claim 35.

37. The *E. coli* cell of claim 36 which further contains a recombinant expression system for a holo ACP synthase.

15

38. A method to produce a polyketide synthase activity which method comprises culturing the *E. coli* cell of claim 36 under conditions wherein expression is favored.

20

39. A method to produce a polyketide synthase activity which method comprises culturing the *E. coli* cell of claim 37 under conditions wherein expression is favored.